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of Army Medical College, 31 Mar 42

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Army Medical College Epidemiological Research Report

Section 2 Number 327

Relationship of Supersonic Wave Frequency and Cellulicidal Action

Part 2. Experimentation with Typhoid Bacillus and Typhoid
and Typhoid Vi Bacillus

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General

The numerous works of KATAHARA¹, YEN and SIN LIN², SAHARA³, CHAI⁴ and FUJIMOTO⁵ contain accounts of the mechanical destruction of typhoid bacilli by means of a supersonic wave treatment and the resulting decrease in toxins and increase in antigenic strength. However, the influence exerted on these bacterial cells by supersonic waves of varying frequencies but of the same energy level has not been reported.

My aim was to further improve the quality of typhoid vaccines through a study of the destructive strength of supersonic waves of varying frequencies on the same antigen.

Chapter I. Outline of experiment

Following the procedure in the cholera bacteria experiment in Part I of this series, the survival, bacterial count, morphological changes and turbidity of typhoid bacilli (Shōdebata strain) and typhoid Vi bacilli (No. 27) were examined.

Chapter II. Experimental procedure

A. Bacterial strain: The typhoid bacilli were obtained from the strain preserved by this laboratory; the Vi bacilli (No. 27) were contributed by Technician KOGIMA of the Infectious Diseases Research Institute. Both strains indicated uniform turbidity in bouillon; gas formation in lactose and dextrose vertical agar was negative; whey culture and milk coagulation were negative; lead acetate agar culture was positive; indol reaction was negative; and the agglutination titer of the typhoid immune serum was positive at 6,400 times.

B. Bacterial suspension: Numerous cultures of Vi bacilli were made since each 3 per cent agar culture (PH 7.4) produced only a small quantity of colonies at 37° C over a 20-hour period. After weighing, a 10 mg per cc solution was prepared with a physiological saline solution; this was diluted to 1.0 mg per cc and 0.1 mg per cc.

Each of the prepared bacterial solutions was cultured in bouillon and agar; tests for miscellaneous bacteria were conducted after preparing smear specimens. Bacterial counts were performed before each experiment since the same bacterial suspensions were not employed whenever the supersonic wave frequency was changed.

C. Supersonic wave treatment: As already related in Part I of this series, the plate current was regulated to produce a test tube temperature (internal) rise of 10° C per minute at each frequency in order to maintain a constant energy input into a test tube containing a specific volume of transformer oil. The following currents were used to provide a uniform energy level:

<u>Frequency</u>	<u>Plate current</u>	<u>Grid current</u>	<u>Plate voltage</u>
1120 kc	460 ma	166 ma	3000 v
560 kc	550 ma	130 ma	3000 v
280 kc	400 ma	120 ma	3300 v

As in the preliminary tests on cellulicidal strength and as outlined in Part I, the treatment time at 1120, 560 and 280 kc was

1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 75, 90, 105 and 120 minutes.

D. Qualitative tests on cellulicidal strength: Two platinum loop portions each of the supersonic wave-treated bacterial suspensions were slant-cultured with agar and plate-cultured with bouillon and agar. After incubating at 37° C for 24 hours the results were evaluated as +++, ++, + and - according to their growth.

E. Bacterial count: The supersonic wave-treated bacterial solutions were diluted progressively ten times each with a physiological saline solution. One cc of each was mixed well with agar (45° C) and allowed to solidify. Agar was added again in an amount sufficient to cover the surface. Bacterial count was taken after incubating at 37° C for 24 hours.

Because the purpose of this test was to determine the number of destroyed bacteria, counts were taken on the control and also during the intermediate stage and the stage immediately prior to destruction. The rate of time decrease compared to the bacterial count of the control was examined. In brief, the percentage of developed colonies per milligram in comparison to the control was determined.

F. Observation of morphological changes: Immediately following the supersonic wave treatment, a smear specimen was prepared with one platinum loop portion of the test solution. Staining with methylene blue was accomplished when this was dried and fixed. Microscopic examinations were made in order to study the nature of the destroyed bacteria.

G. Measurement of turbidity: The bacterial suspensions were diluted according to the ratios shown below with a physiological saline solution and measured with a Pulfrich's photometer. The absolute turbidity was computed from the relative turbidity.

<u>Bacterial solution concentration</u>	<u>Dilution</u>
0.1 mg/1.0 cc	Stock solution
1.0 mg/1.0 cc	10 times
10.0 mg/1.0 cc	100 times

Chapter III. Results of experiment

The results are shown in Tables 1-6. The bacteria survival times are shown in Figures 1-6, the morphological changes in Tables 7 and 8 and turbidities in Tables 1-6.

Table 1. Test results on cellulicidal strength; survival and turbidity of typhoid bacilli treated with 1120 kc supersonic waves.
 Plate voltage 3000 v; plate current 460 ma; grid current 165 ma. Date of experiment 15 Apr 41. Room temperature 26° C. Cloudy.

Plate voltage 3000 v; plate current 450 ma; grid current 165 ma. Date of experiment 15 Apr 41. Room temperature 26° C. Cloudy.																				
0.1 mg							1.0 mg							10.0 mg						
Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 0.1 mg (Z)	Survival compared to control (%)	Log Z	Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 1.0 mg (Z)	Survival compared to control (%)	Log Z	Time (Min.)	Survival of bacteria		Turbidity	Bacterial count - 10.0 mg (Z)	Survival compared to control (%)	Log Z
	Bouillon	Agar slant						Bouillon	Agar slant						Bouillon	Agar slant				
0	++	++	0.0151	70.1 x 10 ⁶		7.8452	0	++	++	0.141	701.2 x 10 ⁶		8.8452	0	++	++	1.092	7.012 x 10 ⁶		9.8452
1	++	++	0.0161				1	++	++	0.151				1	++	++	1.026			
2	+	+	0.0177				2	++	++	0.160				2	++	++	0.803			
3	+	+	0.0195	0.62 x 10 ⁶	0.008	5.7924	3	++	++	0.166				3	++	++	0.780			
4	-	-	0.0206				4	++	++	0.168				4	++	++	0.747			
5	-	-	0.0218				5	++	++	0.195				5	++	++	0.685			
6	-	-	0.0242				6	++	++	0.206				6	++	++	0.658			
7	-	-	0.047				7	+	+	0.214				7	++	++	0.635			
8	-	-	0.0359				8	+	+	0.223				8	++	++	0.613			
9	-	-	0.0265				9	+	+	0.227	9.75 x 10 ⁶	1.39	6.9890	9	++	++	0.602			
10	-	-	0.0268				10	+	+	0.237	2.00 x 10 ⁶	0.280	6.3010	10	+	+	0.580			
							15	-	-	0.4127				20	+	+	0.452	31.100 x 10 ⁶	0.544	7.5912
							20	-	-	0.4015				40	-	-	0.6320	0.9 x 10 ⁶	0.013	5.9543
														50	-	-	0.5465			
														60	-	-	0.7857			
														75	-	-	0.3903			
														90	-	-	0.6803			

Table 2. Test results on cellulicidal strength; survival and turbidity of typhoid bacilli treated with 560 kc supersonic waves.

Plate voltage 3000 v; plate current 550 ma; grid current 130 ma. Date of experiment 4 May 41. Room temperature 29° C.

0.1 mg							1.0 mg							10.0 mg						
Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 0.1 mg (2)	Survival compared to control (%)	Log 2	Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 1.0 mg (2)	Survival compared to control (%)	Log 2	Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 10.0 mg (2)	Survival compared to control (%)	Log 2
	Bouillon	Agar slant						Bouillon	Agar slant						Bouillon	Agar slant				
8	+++	+++	0.01454	72.382×10^6	7.8596	7.8596	8	+++	+++	0.10819	723.824×10^6		8.8596	8	+++	+++	1.05405	7238.240×10^6		9.8596
1	+++	+++	0.01328				1	+++	+++	0.09905				1	+++	+++	1.00386			
2	+++	+++	0.01215				2	+++	+++	0.08867				2	+++	+++	0.97040			
3	+++	+++	0.01199				3	+++	+++	0.08421				3	+++	+++	0.97040			
4	+++	+++	0.01136				4	+++	+++	0.08254	324.213×10^6	44.792	8.5108	4	+++	+++	0.86220			
5	+++	+++	0.01113				5	+++	+++	0.08031				5	+++	+++	0.79193			
6	++	++	0.01037				6	+++	+++	0.07910				6	+++	+++	0.70270			
7	++	++	0.00937				7	+++	+++	0.06915				7	+++	+++	0.65809			
8	+	+	0.00970	10.234×10^6	7.0098	7.0098	8	+++	+++	0.07529	184.349×10^6	25.469	8.2653	8	+++	+++	0.60232			
9	+	+	0.00976				9	++	++	0.06581				9	+++	+++	0.60232			
10	+	+	0.00976	0.296×10^6	0.409	5.4713	10	++	++	0.06358	78.644×10^6	10.312	7.8729	10	+++	+++	0.68559			
15	—	—	0.00926				15	++	++	0.06246				20	+++	+++	0.47405	300.840×10^6	4.156	8.4771
20	—	—	0.00898				20	++	++	0.05911	13.805×10^6	1.907	7.1359	30	+++	+++	0.42985	278.220×10^6	3.857	8.4443
							30	—	—	0.04992				40	+++	+++	0.35693	41.620×10^6	0.575	7.6193
														50	++	++	0.35693	22.620×10^6	0.362	7.3565
														60	+	+	0.77366	2.314×10^6	0.032	6.3643
														70	+	+	0.35693	1.664×10^6	0.023	6.2212
														90	+	+	0.29008	0.271×10^6	0.004	5.4330
														105	—	—	0.25097			
														120	—	—	0.44873			

Table 3. Test results on cellulicidal strength; survival and turbidity of *typhoid bacilli* treated with 330 kc supersonic waves.
Plate voltage 3300 v; plate current 400 ma; grid current 190 ma. Date of experiment 3 Feb 41. Room temperature 31° C.

0.1 mg							1.0 mg							10.0 mg						
Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 0.1 mg (1)	Survival compared to control (%)	log 2	Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 1.0 mg (1)	Survival compared to control (%)	log 2	Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 10.0 mg (1)	Survival compared to control (%)	log 2
	Douillon	agar slant						Douillon	agar slant						Douillon	agar slant				
0	+++	+++	0.01112	71.513×10^6		9.8664	0	+++	+++	0.09035	735.1340×10^5		6.8664	0	+++	+++	0.92578	7351.340×10^4		9.8664
1	+++	+++	0.01382				1	+++	+++	0.09680				1	+++	+++	0.71385			
2	+++	+++	0.01174				2	+++	+++	0.09608				2	+++	+++	0.55770			
3	+++	+++	0.00993				3	+++	+++	0.09443	124.134×10^5	1.444	6.6257	3	+++	+++	0.47962			
4	+++	+++	0.00881				4	+++	+++	0.04908				4	+++	+++	0.41270			
5	++	++	0.00761	11.0893×10^6	15.385	7.0444	5	+++	+++	0.09019	7.083×10^5	0.307	6.8945	5	+++	+++	0.38146			
6	-	-	0.00792				6	+++	+++	0.05342				6	+++	+++	0.35917			
7	-	-	0.00781				7	+++	+++	0.05331				7	+++	+++	0.38443			
8	-	-	0.00768				8	+++	+++	0.05186	1.384×10^5	0.117	6.1093	8	+++	+++	0.36770			
9	-	-	0.00736				9	+++	++	0.05682				9	+++	+++	0.27327			
10	-	-	0.00736				10	+++	++	0.04449	0.187×10^5	0.006	6.6175	10	+++	+++	0.22866			
							15	-	-	0.04409				15	++	++	0.36116	345.399×10^4	0.470	6.5382
							20	-	-	0.06692				20	++	++	0.27397	0.980×10^5	0.001	5.9912
							25	-	-	0.05688				25	++	++	0.26212	0.961×10^5	0.002	5.8068
							30	-	-	0.07250				30	++	++	0.23981	0.823×10^5	0.00003	4.3434
														40	-	-	0.23433			

Table 4. Test results on cellulicidal strength; survival and turbidity of typhoid VI bacilli treated with IL20 by superoxide waves.
Plate voltage 3000 v; plate current 460 ma; grid current 165 ma. Date of experiment 19 Apr 41. Room temperature 21.5° C.

0.1 mg						1.0 mg						10.0 mg								
Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 0.1 mg (x)	Survival compared to control (%)	Log 1	Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 1.0 mg (x)	Survival compared to control (%)	Log 1	Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 10.0 mg (x)	Survival compared to control (%)	Log 1
	Boillon	Agar slant						Boillon	Agar slant						Boillon	Agar slant				
0	+++	+++	0.017	7.31×10^6		7.6639	0	+++	+++	0.153	731.0×10^4		8.6639	0	+++	+++	1.634	$7310. \times 10^6$		9.8631
1	+++	+++	0.016				1	+++	+++	0.128				1	+++	+++	1.450			
2	++	++	0.011				2	++	++	0.125				2	+++	+++	1.385			
3	++	++	0.016	7.27×10^6	99.374	7.6615	3	+	+	0.120				3	+++	+++	0.935			
4	+	+	0.016				4	+	+	0.120				4	+++	+++	0.918			
5	+	+	0.015	3.22×10^6	44.049	7.6076	5	+	+	0.112				5	+++	+++	0.826			
6	+	+	0.015				6	+	+	0.102				6	+++	+++	0.772			
7	+	+	0.015				7	+	+	0.090				7	++	++	0.730			
8	+	+	0.015	3.95×10^6	5.425	6.5966	8	+	+	0.084	1.42×10^6	1.0507	6.6837	8	++	++	0.661			
9	-	-	0.014				9	+	+	0.081				9	++	++	0.642			
10	-	-	0.013				10	+	+	0.073	6.42×10^6	0.7502	6.6420	10	+	+	0.606			
							15	-	-	0.077				15	+	+	0.616	$3195. \times 10^6$	0.4376	9.535
							20	-	-	0.091				20	+	+	0.611			
							30	-	-	0.090				30	+	+	0.601	0.7×10^6	0.002074	5.8451
														35	-	-	0.767			
														45	-	-	1.119			

Table 5. Test results on cellulicidal strength; survival and turbidity of typhoid Vi bacilli treated with 560 kc supersonic waves.

Plate voltage 3000 v; plate current 550 ma; grid current 130 ma. Date of experiment 2 - 5 Jun 41. Room temperature 26° C.

0.1 mg							1.0 mg							10.0 mg						
Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 0.1 mg (x)	Survival compared to control (%)	Log Z	Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 1.0 mg (x)	Survival compared to control (%)	Log Z	Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 10.0 mg (x)	Survival compared to control (%)	Log Z
	Bouillon	Agar slant						Bouillon	Agar slant						Bouillon	Agar slant				
K	+++	+++	0.01174	72.447 x 10 ⁶		7.8599	1	+++	+++	0.08929	724.459 x 10 ⁶		8.8599	K	+++	+++	0.83655	7244.79 x 10 ⁶		9.8599
1	+++	+++	0.01126				1	+++	+++	0.08700				1	+++	+++	0.80309			
2	+++	+++	0.01226				2	+++	+++	0.07919				2	+++	+++	0.73759			
3	++	+	0.01312				3	+++	+++	0.07752				3	+++	+++	0.59116			
4	++	++	0.01360				4	+++	+++	0.07585				4	+++	+++	0.63578			
5	++	++	0.01382	39.357 x 10 ⁶	54.326	7.5949	5	+++	+++	0.07562	317.147 x 10 ⁶	29.973	8.3367	5	+++	+++	0.64693			
6	++	++	0.01430				6	+++	+++	0.06748				6	+++	+++	0.69039			
7	++	++	0.01582				7	+++	++	0.07362				7	+++	+++	0.68039			
8	+	+	0.01582	6.683 x 10 ⁶	9.225	6.8250	8	++	++	0.07473	117.536 x 10 ⁶	16.228	8.0701	8	+++	+++	0.60232			
9	+	+	0.01582				9	++	++	0.07429				9	+++	+++	0.57443			
10	+	+	0.01560	0.115 x 10 ⁶	0.160	5.0607	10	++	+	0.06525	49.736 x 10 ⁶	6.284	7.4778	10	++	++	0.58001			
15	+	+	0.01366	0.33 x 10 ⁶	0.046	4.5185	15	++	+	0.06915	39.471 x 10 ⁶	9.448	7.5963	20	++	++	0.47962	274.83 x 10 ⁶	3.793	8.4391
20	-	-	0.012475				20	++	+	0.06692	33.589 x 10 ⁶	4.623	7.5305	30	++	++	0.41838	15.00 x 10 ⁶	0.200	7.1761
							30	-	-	0.07027				40	++	++	0.44951	11.46 x 10 ⁶	0.162	7.0705
							40	-	-	0.061347				50	++	++	0.06251	6.03 x 10 ⁶	0.085	6.7784
														60	++	++	0.31347	2.55 x 10 ⁶	0.035	6.4065
														75	+	+	0.33693	0.13 x 10 ⁶	0.002	5.1139
														90	+	+	0.29558	0.09 x 10 ⁶	0.001	4.9542
														105	+	+	0.25456	0.012 x 10 ⁶	0.0001	4.0792
														120	-	-	0.15096			

Table 6. Test results on colloidal strength; survival and turbidity of typhoid Vi bacilli treated with 100 kc ultrasonic waves.
Plate voltage 3300 v; plate current 400 ma; grid current 190 ma. Date of experiment 5 Jul 41. Room temperature 31° C.

0.1 mg							1.0 mg							10.0 mg						
Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 0.1 mg (x)	Survival compared to control (%)	Log z	Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 1.0 mg (x)	Survival compared to control (%)	Log z	Time (min.)	Survival of bacteria		Turbidity	Bacterial count - 10.0 mg (x)	Survival compared to control (%)	Log z
	Bouillon	Agar slant						Bouillon	Agar slant						Bouillon	Agar slant				
8	+++	+++	0.1360	73.174 x 10 ⁶		7.8643	8	+++	+++	0.39031	731.341 x 10 ⁶		8.8943	8	+++	+++	0.9481	7317.414 x 10 ⁶		9.8643
1	+++	+++	0.01487	65.935 x 10 ⁶	90.107	7.8191	1	+++	+++	0.06477	79.720 x 10 ⁶	10.893	7.9016	1	+++	+++	0.8120			
2	+++	+++	0.01253				2	+++	+++	0.37440				2	+++	+++	0.6469			
3	+++	+++	0.01015	50.441 x 10 ⁶	68.999	7.6999	3	+++	+++	0.06220	78.830 x 10 ⁶	10.771	7.8967	3	+++	+++	0.5977			
4	+++	+++	0.01048				4	+++	+++	0.06004				4	+++	+++	0.5298			
5	+++	+++	0.00959	45.126 x 10 ⁶	63.836	7.6544	5	+++	+++	0.26413	50.681 x 10 ⁶	8.934	7.6775	5	+++	+++	0.5520			
6	+++	+++	0.01004				6	+++	+++	0.05186				6	+++	+++	0.4673			
7	+++	+++	0.00940				7	+++	+++	0.04135				7	+++	+++	0.4450			
8	++	++	0.00786	11.539 x 10 ⁶	15.769	7.0618	8	+++	+++	0.05800	1.818 x 10 ⁶	1.666	6.0856	8	+++	+++	0.4450			
9	-	-	0.00781				9	+++	++	0.05855				9	+++	++	0.4350			
10	-	-	0.00968				10	++	++	0.06079	0.134 x 10 ⁶	0.004	5.0156	10	+	+	0.3825			
							15	+	+	0.04700				15	+	+	0.2900	3145.009 x 10 ⁶	42.990	6.4962
														20	+	+	0.2768	1065.635 x 10 ⁶	25.469	9.2702
														30	+	+	0.2230	2.341 x 10 ⁶	0.032	6.3694
														40	-	+	0.1617	0.150 x 10 ⁶	0.001	5.1987
														50	-	-	0.1520			
														60	-	-	0.3427			

A. Cellulicidal time.

0.1 mg/1.0 cc 1.0 mg/1.0 cc 10.0 mg/1.0 cc

Typhoid	1120 kc	4 min.	15 min.	50 min.
Bacilli	560 kc	15 min.	30 min.	105 min.
	280 kc	5 min.	15 min.	40 min.
Typhoid VI	1120 kc	9 min.	15 min.	60 min.
Bacilli	560 kc	20 min.	35 min.	120 min.
	280 kc	9 min.	30 min.	50 min.

The results show an acceleration in cellulicidal time (in the order of 280 kc and 1120 kc) with higher bacterial solution dilutions at the same supersonic wave energy level. Destruction, however, is retarded at 560 kc. The cellulicidal times for the typhoid bacilli at 280 kc are six minutes for 0.1 mg, 15 minutes for 1.0 mg and 40 minutes for 10.0 mg. At 1120 kc the time for 0.1 mg is four minutes or a somewhat faster rate than that at 280 kc; the other times are slower. The times for the VI bacilli at 280 kc are nine minutes for 0.1 mg, 30 minutes (15 minutes at 1120 kc) for 1.0 mg and 50 minutes for 10.0 mg. Consequently, the destructive strength at 280 kc is directly proportionate to the frequency and inversely proportionate to the wavelength and is subject to some amount of fluctuation.

B. Survival test: (See Figures 1 to 6) The graphs show that a supersonic wave treatment produces a logarithmic decrease in bacterial count. By taking the bacterial counts in the graphs as logarithmic scales (vertical axes) and the treatment times as arithmetical scales (horizontal axes), approximately straight lines are formed. A step-like decrease is indicated by the 10.0-mg concentration. By treating the bacterial count as y and the treatment time as x , the relationship

$$\log y = ex, \text{ or}$$

$$y = K e^x$$

$$(e = \text{negative constant})$$

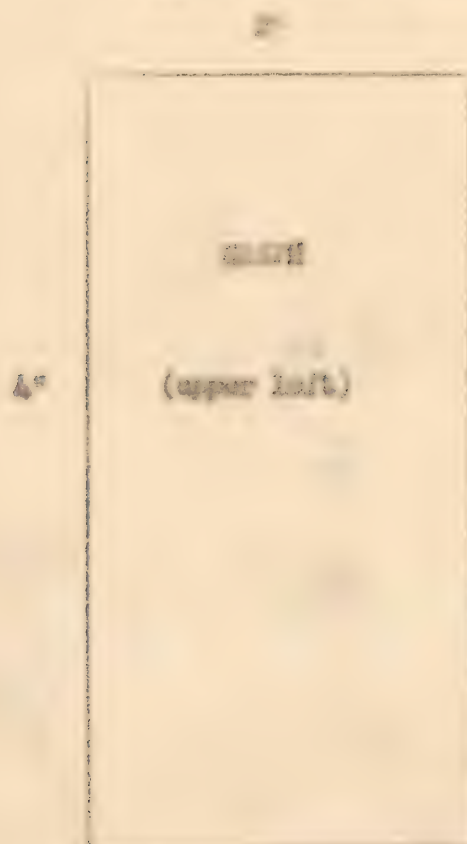
is obtained.

As illustrated by the bacterial counts for the 10.0 mg/1.0 cc concentration in Figures 3 and 6, the decrease in live bacteria count, at the same energy level, becomes more pronounced as the frequency decreases or, in other words, as the wavelength increases.

The time required for the log of the live bacteria count of the 10.0 mg/1.0 cc concentration to become halved is shown below.

Typhoid bacilli	1120 kc	50 min.
	560 kc	75 min.
	280 kc	40 min.
Typhoid VI bacilli	1120 kc	40 min.
	560 kc	90 min.
	280 kc	20 min.

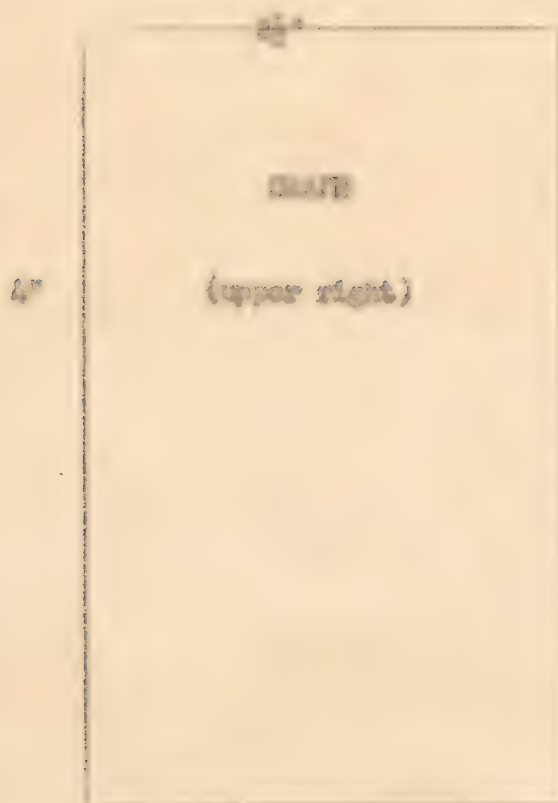
Figure 1. Bacterial count variations
of 0.1 mg/1.0 cc bacterial
(typhoid bacilli) solution



Fig

- 1 Bacterial count.
- 2 Stock solution.
- 3 Time (minutes).

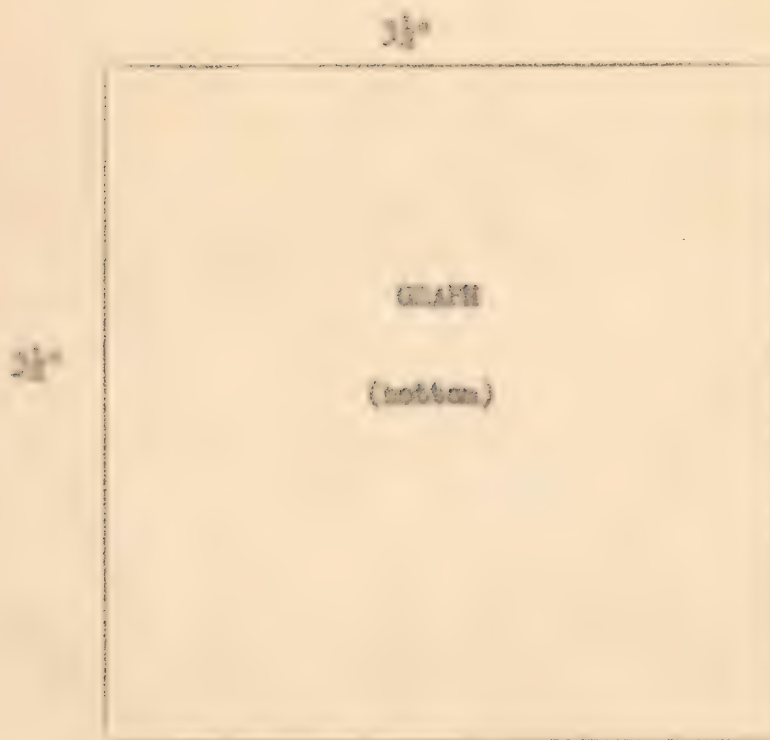
Figure 2. Bacterial count variations
of 1.0 mg/1.0 cc bacterial
(typhoid bacilli) solution



Legend

- 1 Bacterial count.
- 2 Stock solution.
- 3 Time (minutes).

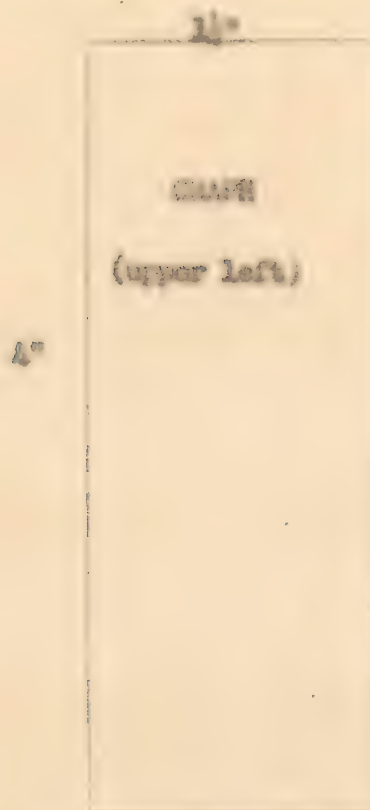
Figure 3. Bacterial count variations
of 10.0 mg/1.0 cc bacterial
(typhoid bacilli) solution



LEG

- 1 Bacterial count.
- 2 Time (minutes).

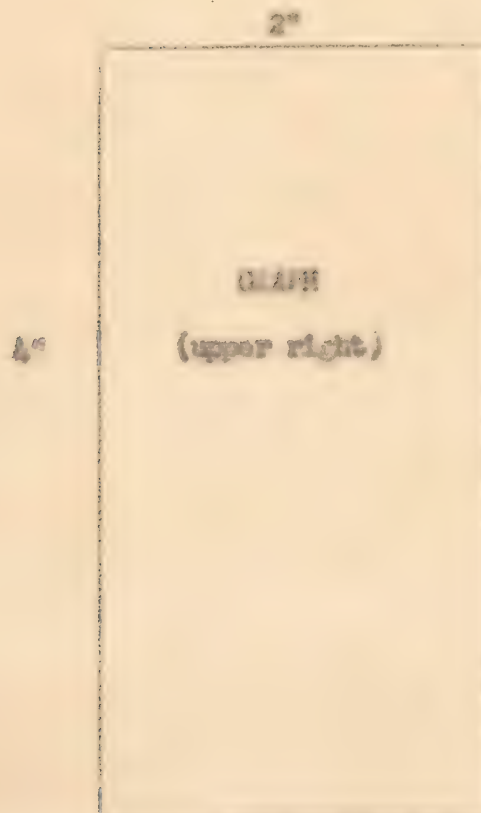
Figure 4. Bacterial count variations
of 0.1 mg/1.0 cc bacterial
(typhoid Vi bacilli) solution



LEG

- 1 Bacterial count.
- 2 Stock solution.
- 3 Time (minutes).

Figure 5. Bacterial count variations
of 1.0 mg/1.0 cc bacterial
(typhoid VI bacilli) solution



LEG

- 1 Bacterial count.
- 2 Stock solution.
- 3 Time (minutes).

Figure 6. Bacterial count variations
of 10.0 mg/1.0 cc bacterial
(typhoid VI bacilli) solution



KEY

- 1 Bacterial count.
- 2 Stock solution.
- 3 Time (minutes).

6. Observation of morphological changes:

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Table 2. Microscopic observations of morphological changes in typhoid Vi bacilli at various supersonic wave frequencies

Frequency			1120 kc									560 kc									280 kc											
Microscopic observations			Cellular swelling	Cellular deformation	Cellular destruction	Protoplasm (homogenization)	Granular	Cloudy	Dust-like	Normal	Inability to stain	Cellular swelling	Cellular deformation	Cellular destruction	Protoplasm (homogenization)	Granular	Cloudy	Dust-like	Normal	Inability to stain	Cellular swelling	Cellular deformation	Cellular destruction	Protoplasm (homogenization)	Granular	Cloudy	Dust-like	Normal	Inability to stain			
Bacterial solution concentration	0.1 mg	Time																														
		K	—	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	##	—		
		1	—	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	##	—		
		2	—	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	##	—		
		3	+	—	+	—	—	—	—	—	##	—	+	—	+	—	—	—	—	—	—	+	—	+	—	—	—	—	—	##	—	
		4	+	—	+	—	—	—	—	—	##	—	+	—	+	—	—	—	—	—	—	+	—	+	—	—	—	—	—	##	—	
		5	+	—	+	—	—	—	—	—	##	—	+	—	+	—	—	—	—	—	—	+	—	+	—	—	—	—	—	##	—	
		6	+	—	+	—	—	—	—	—	##	—	+	—	+	—	—	—	—	—	—	+	—	+	—	—	—	—	—	##	—	
		7	+	+	+	—	—	—	—	—	##	—	+	—	+	—	—	—	—	—	—	+	—	+	—	—	—	—	—	—	##	—
		8	+	+	+	—	—	—	—	—	##	—	+	—	+	—	—	—	—	—	—	+	—	+	—	—	—	—	—	—	##	—
		9	+	+	##	—	—	—	—	—	—	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		10	+	—	##	—	—	—	—	—	—	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		15																														
		20																														
1.0 mg	K	—	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	##	—		
	1	—	—	—	—	—	—	—	—	##	—	+	—	+	—	—	—	—	—	—	+	—	+	—	—	—	—	—	##	—		
	2	—	—	—	—	—	—	—	—	##	—	+	—	+	—	—	—	—	—	—	+	—	+	—	—	—	—	—	##	—		
	3	—	—	—	—	—	—	—	—	##	—	+	—	+	—	—	—	—	—	—	+	—	+	—	—	—	—	—	##	—		
	4	—	—	+	—	—	—	—	—	##	—	+	—	+	—	—	—	—	—	—	+	—	+	—	—	—	—	—	##	—		
	5	—	—	+	—	—	—	—	—	##	—	+	—	+	—	—	—	—	—	—	+	—	+	—	—	—	—	—	##	—		
	6	—	—	+	—	—	—	—	—	##	—	+	—	+	—	—	—	—	—	—	+	—	+	—	—	—	—	—	##	—		
	7	—	—	+	—	—	—	—	—	##	—	+	—	+	—	—	—	—	—	—	+	—	+	—	—	—	—	—	##	—		
	8	+	+	+	—	—	—	—	—	##	—	+	—	+	—	—	—	—	—	—	+	—	+	—	—	—	—	—	—	—	—	
	9	+	+	+	—	—	—	—	—	##	—	+	—	+	—	—	—	—	—	—	+	—	+	—	—	—	—	—	—	—	—	
	10	+	+	+	—	—	—	—	—	##	—	+	—	+	—	—	—	—	—	—	+	—	+	—	—	—	—	—	—	—	—	
	15	+	+	+	—	—	—	—	—	##	—	+	—	+	—	—	—	—	—	—	+	—	+	—	—	—	—	—	—	—	—	
	20	+	+	+	—	—	—	—	—	##	—	+	—	+	—	—	—	—	—	—	+	—	+	—	—	—	—	—	—	—	—	
	30	+	—	##	—	—	—	—	—	—	—	+	—	+	—	—	—	—	—	—	+	—	+	—	—	—	—	—	—	—	—	
40											—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
10.0 mg	K	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	##	—		
	1	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	##	—		
	2	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	##	—		
	3	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	##	—		
	4	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	##	—		
	5	+	—	+	—	—	—	—	##	—	—	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	—	—	—	
	6	+	—	+	—	—	—	—	##	—	—	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	—	—	—	
	7	+	—	+	—	—	—	—	##	—	—	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	—	—	—	
	8	+	+	+	—	—	—	—	##	—	—	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	—	—	—	
	9	+	+	+	—	—	—	—	##	—	—	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	—	—	—	
	10	+	+	+	—	—	—	—	##	—	—	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	—	—	—	
	20	+	+	+	—	—	—	—	##	—	—	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	—	—	—	
	30	+	+	+	—	—	—	—	##	—	—	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	—	—	—	
	40	+	+	+	—	—	—	—	##	—	—	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	—	—	—	
50	+	+	+	—	—	—	—	##	—	—	—	—	—	—	—	—	—	##	—	—	—	—	—	—	—	—	—	—	—	—		
60	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
75	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
90											—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
105											—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
120											—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
135											—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		

Condensation of results from Tables 7 and 8

Frequency (mc)	bacterial weight (mg)	1120				560				280			
		0.1	1.0	10.0	0.1	1.0	10.0	0.1	1.0	0.1	1.0	10.0	0.1
Items observed	1. Normal survival period (min.)	-	-	5	1	7	40	6	6	6	30		
	2. Inability to stain	-	7	15	1	1	2	2	3	3	30		
	3. Cellular swelling	-	6	9	1	1	1	1	1	1	10		
	4. Morphological changes	-	10	10	-	-	8	-	1	1	2		
	5. Cellular destruction	2	5	10	-	7	15	2	3	3	9		
	6. Homogenization of protoplasm	-	4	4	1	1	1	1	1	1	10		
	7. Destroyed matter - granular cloudy dust-like	2	7	30	6	4	4	-	6	-	20		
Typhoid VI bacteria	1. Normal survival period (min.)	8	10	50	10	30	105	8	15	15	40		
	2. Inability to stain	-	-	4	1	1	5	3	3	3	2		
	3. Cellular swelling	2	8	6	2	1	2	1	1	1	3		
	4. Morphological changes	7	8	8	-	-	-	-	7	7	8		
	5. Cellular destruction	2	4	5	2	1	2	1	1	1	1		
	6. Homogenization of protoplasm	-	8	9	-	10	7	-	7	7	8		
	7. Destroyed matter - granular cloudy dust-like	5	9	8	-	15	60	-	1	1	6		
		8	-	16	-	-	75	1	1	1	-		
		2	4	5	2	4	2	1	1	1	4		

It was learned from the foregoing results that the optimum for cellular destruction is at 280 kc for both types of bacilli.

D. Measurement of turbidity: As shown in Tables 1 to 5, transparency was not observed among suspensions of typhoid bacilli within the supersonic wave frequency range covered by this experiment. An extremely slight turbidity decrease was detected in the 10.0 mg Vi bacilli but changes in the others seemed to be non-existent.

Chapter IV. Summary and conclusions.

The following conclusions were derived upon observing the cellulicidal time, bacterial count and turbidity and upon making microscopic studies on cellular destruction of typhoid bacilli and Vi bacilli, the suspensions of which were treated on the same energy level with supersonic waves at frequencies of 1120, 560 and 280 kc.

A. At the same supersonic wave energy level the most effective cellulicidal time is indicated at 280 kc and 1120 kc, in that order. Cellulicide at 560 kc is slow. This action is directly proportionate to the frequency (inversely proportionate to the wave length) in concentrated bacterial solutions and appears to be subject to some amount of fluctuation.

B. A slightly greater amount of difficulty is encountered in destroying the Vi bacilli compared to the typhoid bacilli.

C. The cellulicidal time decreases as the bacterial concentration is lessened.

D. The changes produced in live bacilli, when based on treatment time, can be expressed logarithmically and possess roughly the following relationship:

$$\log Y = cx.$$

Y = live bacteria count

x = treatment time

c = negative constant

E. A microscopic examination of the specimens reveals cellular destruction occurring at 280 kc, 1120 kc and 560 kc, in that order.

F. Transparency does not result: when physiological saline solution suspensions of typhoid bacilli and Vi bacilli (only a slight trace can be noticed in the case of 10.0 mg Vi bacilli) are treated with supersonic waves. No noticeable changes in turbidity are produced.

G. Cellular destruction cannot be gauged by changes in turbidity.

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REF NO. <i>Med 2-1</i>	UNCLASSIFIED	COUNTRY <i>Japan</i>
REPORT NO. <i>TR-839-50</i>	WDGS - INTELLIGENCE REPORT	I.D. NO. <i>694327</i>
SUBJECT: <i>Experimentation with Typhoid Bacillus and Typhoid Vi Bacillus</i>		
FROM: <i>T/I, G-2, GHQ, FEC</i> REFERENCES:		
EVALUATION:	DATE OF INFORMATION: <i>1942</i>	DATE OF REPORT: <i>16 Aug 1950</i>
INCL one	PREPARED BY: <i>ATIS, G-2, GHQ, FEC</i>	SOURCE: <i>Former Japanese Army Medical College</i>

SUMMARY OR SID REPORT:

Forwarded herewith four (4) copies Allied Translator and Interpreter Section Document No. 55388B, "Experimentation with Typhoid Bacillus and Typhoid Vi Bacillus" by Epidemiology Laboratory of Army Medical College, 31 March 1942.

This report concerns experimental tests, which were made in the laboratories of the former Japanese Army Medical College in 1942, to improve the quality of typhoid vaccines. The study outlines the nature of the experiments, discusses experimental procedures and results and gives a summary and conclusion.

For the Assistant Chief of Staff, G-2:

1 Inclosure (4 cys)
as mentioned above

J. H. Polk
J. H. POLK
Lt Col, GSC
Executive

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